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Coronal microleakage of endodontically treated teeth with intracanal post exposed to fresh human saliva

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ABSTRACT

Objective: The aim of this study was to investigate the coronal microleakage of endodontically treated teeth prepared to receive an intracanal post and teeth with an intracanal post but without a prosthetic crown and exposed to contamination by fresh human saliva. Material and Methods: A mechanical-chemical preparation following the step-back technique was carried out in 35 extracted single-rooted human teeth. The teeth were randomly divided into five groups: G1=root canals instrumented, obturated, and prepared to receive an intracanal post (N=10); G2=root canals with cemented posts but without coronal sealing (N=10); PC1=positive control root canals instrumented and open (N=5); PC2=positive control 2 root canals without instrumentation and open (N=5); and NC=negative control healthy teeth (N=5). The crowns were removed except for the control group of intact teeth. The root canals were obturated and sterilized with cobalt 60 gamma irradiation and were then adapted in an apparatus using a Brain Heart Infusion (BHI) medium and fresh human saliva for contamination. Microbial growth was indicated by the presence of turbidity in the BHI liquid medium. Results: Data were submitted to the Kaplan-Meier Survival Analysis and the Holm-Sidak statistic method, which observed an index of 90% of microleakage in root canals after 24 hours for G1 and 70% of microleakage in samples at the end of 40 days for G2. Conclusion: The results show that root canals with an intracanal post but without a prosthetic crown can be recontaminated when exposed to fresh human saliva in a short period.

Keywords: Endodontics. Saliva. Post and core technique. Dental leakage. Root canal.

INTRODUCTION

The goal of endodontic treatment is to eliminate diseased pulpal tissue and to create an environment that allows periapical tissues to heal and to prevent the development of apical periodontitis. Affected teeth are retained by removing diseased tissue, sealing the canal system, and subsequently restoring the coronal tooth structure[4]. Although many factors of technical order are involved in the failure of endodontic treatment, the emergence of bacteria that resist therapy and proliferate in the root canal, or that contaminate the canal after endodontic treatment through coronal microleakage, is primarily responsible for unsuccessful treatment[17,22].

Frequently, the success of endodontic treatment is associated with an appropriate apical sealing[3,12]. However, the coronal seal reached using restorations has been considered important. In certain situations, the filling can be exposed to the oral environment, as in the case of failures or losses of restorations,

fractures of the dental structure or the restorative material, or during the intraradicular preparation for post placement[9,15]. Furthermore, the prosthetic crown may come off, exposing the intracanal post to the buccal environment.

Theoretically, any bacteria present in the oral cavity can invade the root canal and participate in the installation of an infectious process[21,22]. *Enterococcus faecalis*, bacterium species frequently isolated in root canals with endodontic failure[13,19,21,23], represent a small portion of the initial microbiota of teeth with pulp necrosis and periradicular lesions[6,22]. This bacterium demonstrates resistance to endodontic disinfection procedures during chemical-mechanic preparation[6,19] and the capacity to survive in root canals as a single species without the need for a cooperative relationship with other bacteria[19].

Despite not simulating some conditions of the oral cavity, as the temperature changes, the influence of the diet and saliva flow and the use of human saliva remain the most simulating in a real clinical situation[17].

The dental literature is increasingly recognizing the importance of the coronal seal[2,16,22]. In this context, the importance of an adequate coronal seal in endodontically treated teeth is clear during therapeutic sessions, after filling the root canals, or after placement of the intracanal post and its prosthetic crown.

Therefore, the objective of this study was to investigate the recontamination of root canals of endodontically treated teeth prepared to receive an intracanal post, and those with an intracanal post but without a prosthetic crown and exposed to fresh human saliva. The null hypothesis accepted in this study was that the root canals with intracanal post but without a prosthetic crown can be recontaminated when exposed to fresh human saliva.

MATERIAL AND METHODS

Thirty-five extracted single-rooted human teeth were used in this study. These specimens were obtained from the Teeth Bank of the Pelotas Dental School (Federal University of Pelotas, Brazil, RS) after the Ethics Committee approved this study (Document no. 013/2006). The teeth were stored in 0.9% saline and kept moist at 37°C throughout the experiment. The saline solution was renewed every 7 days until the moment of use. The crowns were removed with a diamond disk in a low-speed handpiece at the level of the enamel-cement junction, except for the teeth in the negative control group, which stayed intact.

The number of selected teeth for the groups was based on previous studies[7]. A mechanical-chemical preparation following the step-back technique was carried out on the 30 extracted single-rooted human teeth. The working length was measured by introducing a #10 K-file (Maillefer, Dentsply Maillefer Ind. e Com. Ltda., Petrópolis, RJ, Brazil) into the root canal and establishing it 1 mm from the root apex. All canals where then instrumented to a size #20 file at working length. To standardize the diameter of the apical foramen, the canals were enlarged to a #20 file. The teeth with foramens larger than #20 were discarded. The instrumentation was completed when the #40 file reached the working length. The coronal portion of the canal was prepared with Gates-Glidden burs #2 and #3. 1 mL of 1% of hypochlorite (NaOCl) (Wirath Indústria e Comércio Ltda, São Paulo, SP, Brazil), followed by 1 mL of 17% of ethylenediaminetetraacetic acid (EDTA) (Iodontosul, Souza e Leonardi Ltda., Porto Alegre, RS, Brazil) were used to irrigate the canals between each file to remove organic and inorganic debris. Then, 1 mL of sterile saline solution was used (Basa, Indústria Farmacêutica Basa Ltda., Caxias do Sul, RS, Brazil) to remove tissular debris, complemented by irrigating solutions (NaOCl and EDTA). All canals were dried with paper points (EndoPoints Indústria e Comércio Ltda., Barão de Angra, Paraíba do Sul, RJ, Brazil) previously sterilized to the diameter of the last file size (#40). The root canals were obturated using a lateral condensation technique with gutta-percha cones of size 40 (as the main cone) previously disinfected with 1% NaOCl for 30 minutes and endodontic cement. The endodontic cement used was EndoFill (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil), prepared to the appropriate consistency following the manufacturer's instructions, and was introduced into the canal together with the main and accessory gutta-percha points (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil).

When the obturation was concluded, the excess material was removed with a heated Paiva condenser along with part of the obturation up to two-thirds of the working length. Approximately 2 mm of obturation were left in the apical third. X-rays were taken (Kodak Ltda., São José dos Campos, SP, Brazil) to verify the quality of the filling and the space for the intracanal post and x-ray images were taken of the root filling (Dabi Atlante, Ribeirão Preto, SP, Brazil) with a radiation exposure time of 0.5 s. The radiographic film was aligned parallel to the long axis of the tooth, and the central beam was directed along the buccal-lingual plane perpendicular to the film. The films were processed manually using the time-temperature method.

The teeth were randomly divided into five groups as follows: group 1 (G1)=10 endodontically treated root canals prepared to receive an intracanal post and group 2 (G2)=10 endodontically treated root canals cemented with a prefabricated intracanal

post without a prosthetic crown. Five teeth with instrumented root canals, opened access cavities, and not obturated served as positive control group 1 (PC1), five teeth with no instrumented root canals and access cavities opened served as positive control group 2 (PC2), and five intact (without endodontic treatment) teeth served as the negative control group (NC) (Figure 1).

The root canals of G2 were etched with 37% phosphoric acid (Dentaltec Ltda., Joinville, SC, Brazil) for 15 s, washed with physiological solution, and dried with sterile absorbent paper points. Following conditioning of the root canal dentin, the Prime & Bond 2.1 adhesive system (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil) was applied according to the manufacturer's instructions. Then, carbon fiber posts (Angelus Indústria de Produtos Odontológicos S/A, Londrina, PR, Brazil) were cemented using resin cement (Cement-Post, Angelus Indústria de Produtos Odontológicos Ltda., Londrina, PR, Brazil) also according to the manufacturer's instructions. All the teeth were kept at 37°C and in 100% relative humidity for 2 weeks to allow the endodontic cement in groups 1 and 2 and the control groups (PC 1, PC 2, and NC) to reach completely cure. All samples were sterilized with radiation using a Cobalt 60 machine (Embrarad Empr. Bras. de Radiações Ltda., Cotia, SP, Brazil).

Test of coronal microleakage

The apparatus used to evaluate the microleakage was prepared according to Siqueira Jr., et al.[17] (2000). A glass tube with rubber stoppers was adjusted for use in this experiment. Using a heated instrument, a perforation was made in the center of every rubber stopper and one tooth was inserted under pressure up to its cement-enamel junction, to ensure that its crown was outside the vial and its root was within the vial. Cylinders prepared from 10 ml sterile plastic syringes were adapted on the external surface of the stoppers to create a chamber around the crown of the tooth. Then, the glass tube was filled with Brain Heart Infusion (BHI, Acumedia Manufacturers, Inc., Lansing, MI, USA) sterile broth and the root apex was immersed in the broth. Cyanoacrylate (Super Bonder®, Henkel Loctite Adesivos Ltda., Itapevi, SP, Brazil) was applied in the interface between the tooth and the stopper to prevent the saliva from penetrating through this space into the BHI broth. One mL of sterile methylene blue (1%) was placed inside every chamber mounted in adapted plastic syringes to assure the efficiency of the sealing of the cyanoacrylate[6]. If the medium becomes blue, then the sealing was ineffective and the specimen needs to be discarded.

A parafilm (American National Can, Chicago, IL, USA) was used to block the interface flask-stopper of the apparatus. The glass tubes were incubated at 37°C for 4 days to ensure sterilization.

Thirty mL of human saliva was collected from a volunteer at 8:00 a.m. every day of the experiment. The volunteer did not engage in oral hygiene for at least 12 hours before the saliva collection. The volunteer chewed a piece of parafilm (1 g) to stimulate salivation. The saliva was stored in a brown 100 mL glass screw-top container, according to Magura, et al.[11] (1991). The chamber of each apparatus was filled with 3 mL of human saliva mixed in BHI broth in a 3:1 (v/v) ratio and with 1 mL of the methylene blue dye solution added. Human saliva was changed every 3 days to ensure that the microorganisms did not lack nutrients[7].

The entire apparatus was aerobically incubated at 37°C and 100% relative air humidity, and the appearance of turbidity in the BHI broth was checked daily for the 40 days. The number of days it took for bacterial growth to appear was indicative of the total recontamination of the root canal by bacteria from saliva. The turbidity was measured visually with the McFarland scale, trying to approximate the turbidity of inocula to the concentration of cells in a suspension. Care was taken with the evaporation of the BHI broth to keep

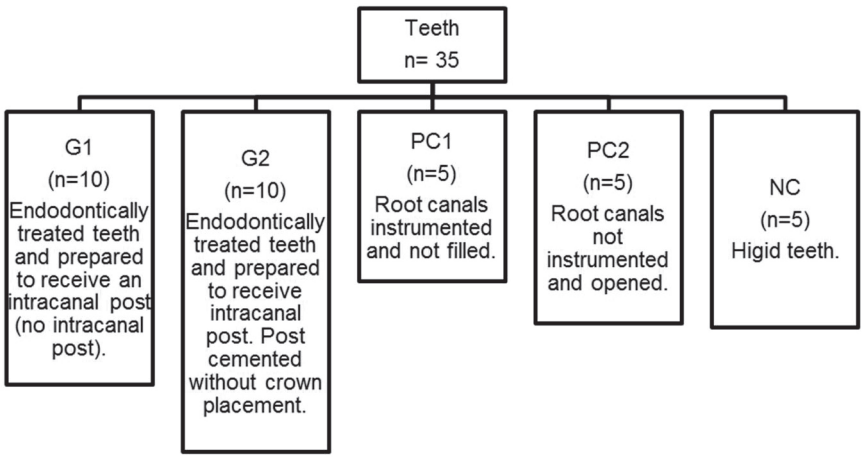

Figure 1- Flowchart of the experimental groups

the roots totally immersed in the medium inside the apparatus during the experiment. Every 48 hours, 4 mL of liquid mixture inside the apparatus chamber were removed and replaced with another 4 mL of sterile BHI broth to avoid saturating the bacterial medium[7]. All procedures were made in a laminar flow under sterile conditions.

Statistical analysis

Data were statistically analyzed using the Kaplan-Meier Survival Analysis test and the Holm-Sidak method. A *P*-value<0.05 was considered statistically significant.

RESULTS

Regarding the possibility of recontamination of the root canals, a statistically significant difference was observed among the groups through the Kaplan-Meier Survival Analysis test (P<0.001) (Figure 2).

This analysis was complemented by the Holm-Sidak method (P=0.05), which showed differences among the groups NC and G1, PC1 and PC2, and PC1 and G2.

An analysis of the statistical average of the recontamination time of each group using the Kaplan-Meier Survival Analysis test verified that no recontamination occurred during the 40 days of the study in the group of intact teeth (NC). In the group of open teeth and not instrumented (PC2), contamination occurred on an average of 1.6 days. In the group of instrumented root canals and not obturated (PC1), contamination occurred on average once a day. In the group of teeth prepared to receive an intracanal post (G1), recontamination occurred on average on 4.9 days, and in the group of root canals that received intracanal posts cemented with resinous cement (G2) but without a prosthetic crown, contamination occurred on an average of 22.4 days (Table 1).

The root canals instrumented, filed, and prepared to receive an intracanal post and later exposed to fresh human saliva (G1) had an index of 90% (N=9) leakage during a period of 24 hours. The group of teeth that received an intracanal post cemented with resinous cement and exposed to fresh human saliva (G2) showed that 20% of the samples were recontaminated in 24 hours and 30% in 48 hours; however, after 14 days, only one more sample showed recontamination. At the end of the 40 days, 70% of the G2 samples were infiltrated (Table 1).

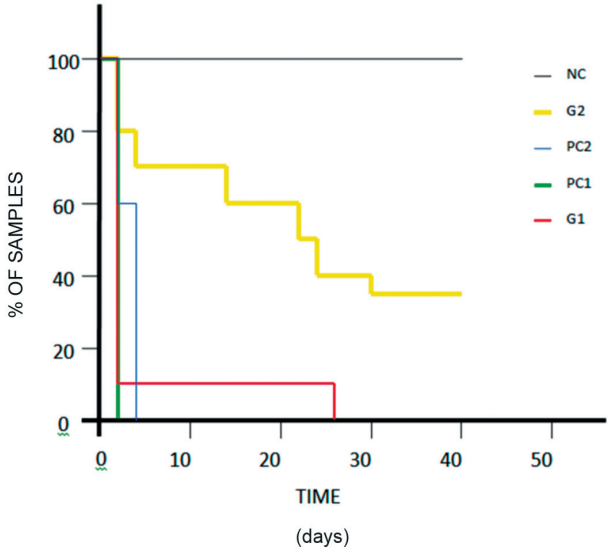

Figure 2- Possibility of recontamination of the root canals - Kaplan-Meier Survival Analysis test (P <0.001)

DISCUSSION

Based on the literature, the presence of recontamination of the root canals exposed to human saliva was typically observed[16], showing the importance of restoration in teeth with a prefabricated intracanal post. Furthermore, the exposure of endodontically treated teeth to the buccal environment may necessitate endodontic retreatment.

This study is in accordance with previous studies reported in the literature[1,2,5,24]. The microorganisms in human saliva can quickly cross the root canal in the absence of coronal sealing, reaching the root apex, and provoking infectious and inflammatory processes in the periradicular tissues[16].

The duration of 40 days for this study was based on the literature[2,7,8,10]. The group of opened and instrumented root canals used as a positive group (PC1) was penetrated by human saliva microorganisms during a period of 24 h and, as

Table 1- Distribution of root canals exhibiting bacterial microleakage after 40 days

Group	Total(N)	No Leakage	Leakage	%	Range(Days)
G1	10	0	10	100	1-26
G2	10	3	7	70	1-29
PC1	5	0	5	100	1
PC2	5	0	5	100	1-3
NC	5	5	0	0	-

expected, proved that root canals exposed to the oral cavity are infected in a short period.

In the group with open teeth and not instrumented (PC2), the time requested for the apical contamination was longer than for the group with open teeth and instrumented (PC1). This result may have been from the instrumentation that provided a more open root canal with the removal of smear-layer and debris, promoting a wider road for the arrival of microorganisms to the root apex without interference, as in teeth not instrumented.

The G1 teeth had an index of 90% of root canal microleakage during a period of 24 h, demonstrating that the 4 mm of remaining filling material did not represent a barrier for the bacterial coronal leakage. The indicative time of turbidity of the BHI broth for G1 was practically the same as that for instrumented root canals without fillings (PC1). According to Timpawat, Amornchat and Trisuwan[20] (2001), most of the endodontic cements have an antibacterial effect that can limit the entrance of bacteria. Certain new sealers, such as Epiphany and GuttaFlow with primer, along with Apexit, showed better resistance to bacterial penetration than other new or traditional sealers tested[20]. However, this phenomenon is not in accordance with the results of this study. Magura, et al.[11] (1991) suggested that the presence of zinc in gutta-percha and the endodontic cement used in their study might have inhibited bacterial penetration and growth. However, this phenomenon was not evident in the present study.

The root canals that received carbon fiber posts cemented with resinous cement experienced a small percentage of bacterial microleakage in the first 24 and 48 hours (20% and 30% sample leakage, respectively). Otherwise, technical failures and microcracks might have helped with this recontamination. The use of resinous cement could not have prevented the leakage but helped to slow it. Furthermore, in 30% of the samples, complete recontamination of the root canals was not evident during the 40 days of the experiment. Despite the advantages of using resinous cements, the procedure is very sensitive to mistakes such as allowing filling material remnants to stay in the pulp chamber, which would create a difficult acid condition for adhesion. In this study, autopolymerized resinous cement was used to guarantee polymerization in the entire extension of the root canal because passing light through the canal is known to be difficult. The evidence from this study shows the importance of the immediate coronal sealing. Definitive restoration should be done preferably as soon as possible, except when the treatment requires a longer period. In this case, an adequate temporary restoration should be done and the root canal should be dressed with an antimicrobial material to reduce bacterial penetration. Additionally, the root canal dressing prevents the presence and proliferation of microorganisms that resist the chemical-mechanic preparation of the root canal and protects the periapex[7].

The findings of this *in vitro* study show that the contamination of a root canal exposed to fresh human saliva is rapid[17,18]. They affirmed that root canals directly exposed to saliva could be quickly recontaminated from the solubilization of the endodontic cement and the permeability of the filling. However, they could not define when the bacterial leakage provokes an event of periradicular infection, because such an event depends on other factors, including the virulence of microorganisms, defense capacity of periradicular tissues, nutrition, and bacterial interactions. However, the presence of microorganisms in the periapex indicates that chronic or sharp lesions can grow[5].

Despite the limitations of the *in vitro* leakage tests, they supply an appropriate initial framework of new filling materials and techniques[1,2,15]. The use of human saliva has an advantage because it is faithfully closer to the real clinical situation. However, it does not simulate similar variables that exist in the buccal environment given temperature changes, the influence of the diet, and salivary flow[8]. Further studies are necessary to investigate the relationship between the leakage of root fillings and the reactions of periradicular tissues[15].

Magura, et al.[11] (1991) evaluated root canals filled and exposed to the oral environment for three months and suggested retreatment of the root canals. This result is in accordance with the results of the present study, in which the recontamination of root canals without the protection of a coronal restoration and complete filling of the root canal occurred in less than one week. Furthermore, 70% of the root canals that received an intracanal post cemented with resin cement experienced recontamination after 29 days. However, clinically determining whether contact between saliva and the periradicular tissues continues to contraindicate the definitive dental restoration of a tooth whose root canals remained exposed to the oral cavity for a short period remains impossible[14]. Hence, new studies are extremely important to estimate when a retreatment is necessary for teeth exposed to the buccal cavity.

CONCLUSION

This study showed that root canals with an intracanal post become recontaminated when exposed to fresh human saliva in a short period.

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